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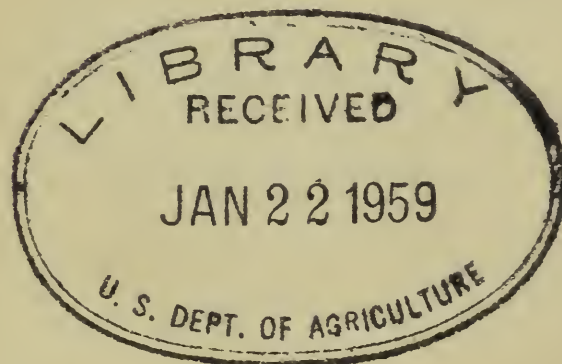
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ENTOMOLOGICAL USES OF RADIOISOTOPES

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CHAPTER 27

ENTOMOLOGICAL USES OF RADIOISOTOPES*

I. INTRODUCTION

During and after World War II studies of atomic energy for defense purposes stimulated a great deal of interest and experimentation in the use of radioisotopes for peaceful purposes. The information obtained in defense research has made available and lowered the cost of hundreds of radioactive materials, which are being used in thousands of ways to further knowledge in industry, agriculture, and medicine.

Considering the importance of insects to man, and to his crops and domestic animals, it is not surprising that entomologists soon recognized that radioisotopes would be very useful in the studies of insects and their control. Insects destroy a sizable part of growing food crops. Infestations reduce yields, increase the cost of production, and lower quality. They torment livestock, thus reducing weight gains and milk yields. Insects attack stored food and other products, man's clothing, household furnishings, and buildings. They take an enormous toll of our forests and indirectly create fire hazards.

In addition, insects are transmitters of some of the most serious diseases of man and animals. Malaria, carried by *Anopheles* mosquitoes, has been a scourge of mankind for thousands of years. This disease has been estimated to kill 2 million persons every year and sicken approximately 100 times this number so that they are unable to perform effective work. Other important insect-borne diseases of man are yellow fever, typhus, and bubonic plague, which cause much suffering and death. Livestock diseases, such as cattle fever, anaplasmosis, encephalitis, anthrax, filariasis, and trypanosomiasis, are carried by insects and cause great economic losses to the livestock industry.

A great number of scientists, including entomologists, chemists, and others, are studying insects and how to control them. The exact number is not known, but the largest entomological association in the United States has approximately 4500 members. Many of these researchers have recognized the importance of radioisotopes in furthering information on biology and control. Common methods employed to combat insects include biological control, cultural practices, mechanical devices,

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drainage and filling, and the employment of chemicals. The use of insecticides is the most widely applied single method of control. Pains-taking research by State, Federal, and industrial scientists employ all these measures in the control of many different kinds of injurious insects. This research is a continuous effort to find more effective and lower cost materials for use in protecting man, his crops, and his livestock.

Recently new problems have arisen which limit the effective and safe use of insecticides. For example, resistance of many species of insects to certain insecticides has made it necessary to intensify research to find substitute chemicals. Much work has also been devoted to seeking an understanding of the nature and cause of insect resistance. Knowledge of residues occurring in plant and animal products following the use of insecticides is necessary to protect the public from undue hazards. Radioisotopes have been used extensively in studies on insect resistance and insecticide residues.

It is the purpose of this author to point out a few of the more significant developments in uses of radioisotopes in entomology during the past several years. Progress in the field has been rapid, and numerous papers are appearing every year. In 1950 Jenkins and Hassett (1) found only 40 references on the subject, but in 1957 there were over 500 papers dealing with the use of radioactivity in entomology. Although there are many fine contributions in this field, only a few can be mentioned here.

II. BIOLOGY

A comprehensive knowledge of their life history, food habits, mating, dispersal, and behavior is essential in studying ways to obtain economic control of noxious insects. Frequently weak links in the biology of a particular pest can be exploited in developing effective chemical or other control procedures. Radioisotopes have contributed in numerous studies on the biology of many species of insects.

Dispersal and flight range of insects have been studied by tagging them with radioisotopes. An appropriate radioisotope is applied directly on the insects, or incorporated in the rearing medium or food in the laboratory or in the natural environment. The tagged insects are later captured and identified with counting equipment. Those having radioactivity can be positively identified weeks or months after treatment, depending on the half-life of the isotope used. Examples of tagging procedures for mosquitoes in ponds to which radioactive phosphorus had been added are recorded by Hassett and Jenkins (2). Reports by Jenkins and Hassett (3), Thurman and Husband (4), Provost (5), and Quartermann et al. (6) show that various species of mosquitoes can be tagged in large numbers and used for flight range and dispersal studies.

TABLE 27-1
DISPERSAL AND FLIGHT OF RADIOACTIVE MARKED FLIES*

Locality	Habitat	Number of flies released ($\times 10^3$)	Per- cent re- covery	Effec- tive dis- persal (miles)	Maxi- mum flight range (miles)	Reference
House fly, <i>Musca domestica</i> (L.)						
Oregon	Rural	36	4.6	1	12	Lindquist et al. (13)
		54	2.78	4	20	Yates et al. (14)
Arizona	City	31	0.73	2	3	Schoof et al. (15)
		56	0.51	5	5	Schoof et al. (15)
		342	1.8	2	9	Schoof and Siverly (16)
Georgia	Rural	13.5	8.0	5	8.25	Quarterman et al. (17)
	City	40	0.24	4	7.6	Quarterman et al. (18)
Blow fly, <i>Phormia regina</i> (Meig.)						
Oregon	Rural	1.2	14.0	4	8	Lindquist et al. (13)
		60	2.64	15	28	Yates et al. (14)
West Virginia	City	16	0.06	5	10.3	Schoof and Mail (19)
Georgia	City	—	—	4	—	Quarterman et al. (18)

* Entire table from Jenkins (7).

Jenkins (7) listed numerous papers on dispersion of insects in his comprehensive review in 1957.

Agricultural and forest insects have been labeled for biological and other studies by Rings and Layne (8), Rings (9), Jones and Wallace (10), Davis and Nagel (11), and Godwin et al. (12), to mention a few. Radioisotopes contributed to the finding of new and useful knowledge in these experiments.

House flies, *Musca domestica* (L.), and blow flies, *Phormia regina* (Meig.), have been tagged for flight studies in several parts of the United States. Some of the data are summarized in Table 27-1. On one occasion blow flies were captured 28 miles from the point of release.

One use of the tagging technique in population studies is to determine the effectiveness of control procedures. The number of insects in a locality can be estimated by releasing known numbers of tagged individuals and then comparing the numbers of tagged and untagged specimens collected in the traps or by other means.

III. RADIATION EFFECTS

27-1 Lethal radiation. Ionizing radiations will kill insects as well as other animal or plant life. There is a wide range of susceptibility of different animals to radiation and much variation between species of insects. The radiations injure cells, particularly the nucleus, by causing physical and chemical changes. The lethal radiation dose for some insects may be 100 times as much as that for man. Plough (20) has estimated the respective LD₅₀'s (50 percent mortality doses) for man, dog, mouse, rabbit, and hamster as 400, 550, 650, 750, and 1000 r, respectively.

To kill insects within a day or two requires a greater dose than when mortality is desired several days after exposure. Hassett and Jenkins (21) found that all *Drosophila* adults were killed in 2 days after exposure to 193,000 r, but 64,400 r was sufficient to cause complete kill in 21 days. Similar results were obtained with the black carpet beetle, *Attagenus piceus* (Oliv.). Species and sex differences in tolerance to radiation have been recorded. Cole et al. (22) showed that all active stages of the American cockroach, *Periplaneta americana* (L.), were killed 24 hours after an exposure to 68,000 r, whereas body lice, *Pediculus humanus* (L.), required 200,000 r. Female German cockroaches, *Blattella germanica* (L.), were all killed by 90,000 r, but males required 98,000 r. Workers of the Pharaoh ant, *Monomorium pharaonis* (L.), required 210,000 r, but the queens were killed by 200,000 r.

Practical use of radiations to destroy insects in stored grains, processed and packaged foodstuffs, clothing, and wood products has been considered for several years. Hassett and Jenkins (21) proposed using low-cost fission products for controlling insects in stored materials. After studying the effects of insects exposed to cobalt-60 and tantalum-182, they concluded that the carpet beetle, *Anthrenus scrophulariae* (L.), cigarette beetle, *Lasioderma serricorne* (F.), and rice weevil, *Sitophilus oryza* (L.), could be killed fairly rapidly by a dose of 65,000 r. Some other insects might require higher or lower doses. Although this method of controlling insects has promise, several problems must be solved before it is put into widespread use. For example, foods containing dead insects may not be acceptable to the consumer, and any technique used to kill insects without removing them from packaged foods will be looked on with disfavor. Another factor is the high cost of radiating large volumes of material. Fumigation, now widely used for the control of stored-product insects, is much less costly.

Much attention has already been given to the use of radiation for destroying harmful bacteria in meat and dairy products. Studies show that some of these foods are changed in color, texture, odor, or flavor

by treatment with ionizing radiations. Similar changes may also follow radiation of stored-food products for insect control. In time these problems may be resolved and radiated foods will then be acceptable.

The use of radiation to destroy insects in bulk grain is more promising. In a review of the present status of stored-product insect control by radiation, Hassett (23) tabulated estimates of costs of different energy sources. One of the better sources was spent reactor fuel elements, which were estimated to be capable of treating 27 tons of material per hour at a cost of 75 cents per ton. Other energy sources may be mixed fission products and cesium-137. The Fission Products Laboratory at the University of Michigan has plans for a unit that can be installed in a boxcar and be capable of processing 6500 bushels of material per day at an estimated cost of 6 to 8 cents per bushel.

Another use of radioisotopes is in the destruction of immature stages of the oriental fruit fly, *Dacus dorsalis* (Hendel). Balock, Christenson, and Burr (24) found that eggs up to 6 hours old were killed by dosages of about 4000 r, but eggs 24 hours of age were unaffected and hatched after exposures up to 36,000 r. Exposures of 120,000 r reduced hatching by 46 percent. Dosages of 2000 r permitted development from egg to adults, which mated and produced normal progeny. Dosages in the 30,000 to 60,000 range permitted only 0.5 percent pupation. Third-instar larvae withstood up to 240,000 r, and approximately 70 percent of them pupated but failed to develop to the adult stage.

These results indicate that the treatment of fresh fruits and vegetables with gamma rays might be an effective and feasible means of destroying insect infestations. Such a method would be of immense value in preventing the introduction of serious pests into countries where they do not now occur.

27-2 Sterilizing radiations. The use of sexually sterilized males as a means of controlling some insects has aroused considerable attention recently. Massive doses of radiations will kill adult insects rather quickly and smaller doses more slowly, but some levels of radiation will not cause any major injury other than sexual sterility. The amount of radiation necessary to cause sexual sterility is much less than required to cause death, reduce longevity, or induce other deleterious effects. For example, Grosch and Sullivan (25) found that only 5000 r made the wasp *Habrobracon* [= *Bracon hebetor* (Say)] sterile, but a massive dose of 180,250 r did not cause death—only a sluggishness from which the wasps recovered. As mentioned earlier, 65,000 r killed several species of stored-products insects, and 16,000 r rendered them incapable of reproduction.

The introduction of sexually sterile insects into a normal population has high potential for the control of some species. The progeny from the mating of a normal female with a sterile male should produce infertile eggs. Geneticists and cytologists have known for many years that insects, especially *Drosophila*, can be made sexually sterile by exposure to x-rays.

The possibility of insect control or eradication through the use of sexually sterile males, especially the screw-worm, *Callitroga hominivorax* (Cqrl.), has been discussed by Knipling (26) (Fig. 27-1). The screw-worm fly lays eggs in wounds of warm-blooded animals, and the eggs hatch into flesh-burrowing larvae (Fig. 27-2). Theoretically, the sustained release of sterile males in numbers exceeding those in a normal population should result in extinction of the population. If the first release of an insect exceeds the native population by five or ten times, the next generation should be greatly reduced, provided the population is reasonably stable. Continued introduction of the same numbers of sterile males will provide higher and higher ratios of sterile to fertile insects, and the species will rapidly become extinct if all circumstances are favorable. The method will not be effective for many species and is likely to be high in cost, but it should prove practicable primarily as an



FIG. 27-1. Male (*left*) and female (*right*) of the screw-worm. (Shown 6 times life-size.)



FIG. 27-2. A large screw-worm infestation in a steer, with newly laid white eggs at the rim of the wound.

eradication tool or for preventing buildup or spread of highly destructive insects that have become established in an area.

Several factors must be considered in an appraisal of sterilization techniques as a means of practical control. An economical method of rearing millions of insects must be available; the insect must be of a type that can readily be dispersed by aircraft or other means; the sterilizing procedure must not harm the mating behavior of the males; and the females should preferably mate only once, but if multiple matings occur the irradiated (sterile) males must produce sperm to compete with sperm of fertile males. The species to be controlled must have a low population, and the area to be treated must, of course, be reasonably protected against reinfestation, preferably isolated by water, mountains, or other barriers. If the population is too high, other methods may be needed to reduce the numbers to feasible levels. Advantage may also be taken of seasonal fluctuations, since most insects are less numerous at some season of the year than at others. Obviously, a thorough knowledge of the habits and ecology of the insect is essential.

The screw-worm appeared to be an ideal insect with which to work, since it meets the requirements mentioned above. The male mates many times but the female only once. Since it is comparatively scarce in



FIG. 27-3. Rearing screw-worm larvae for use in field tests to evaluate sterile-male technique. Medium is kept at 104°F by heating elements under the vats. It consists of lean beef and blood.

nature, usually not over 500 to 1000 per square mile, it appeared possible to rear and release more sterile males than the number present in the field (Fig. 27-3). Information on the ecology, life history, population densities, host relationships, and other data [Lindquist (27)] served as a basis for the consideration of this insect for studies on release of sterile males to effect control or eradication.

The basic laboratory work on the effect of radiation on the screw-worm fly was done by Bushland and Hopkins (28,29). The maximum sterilizing effect with minimum adverse effects on adults was achieved by irradiating pupae. The optimum time was determined to be 5 to 6 days after pupation at 80°F, or 2 or 3 days before adult emergence. A dose of 2500 r caused sterility of males, and 5000 r of females. These doses had no deleterious effect on mating behavior. In cage tests with a 5- or 10-to-1 ratio of sterile to fertile males essentially the same ratio of sterile to fertile egg masses was obtained. Irradiated males competed for the females about equally with normal males, and the biotic potential of a caged population was greatly reduced.

Although a great amount of work was done on various aspects of the screw-worm problem, including small field tests in Florida, the crucial test of theories concerning the effect of introducing sterile males among a natural population was made in 1955. It was necessary to conduct an experiment on an isolated island where screw-worm flies would not reinfest the area. The island chosen was Curaçao, in the Netherlands Antilles, which had a natural infestation similar to that in heavily infested parts

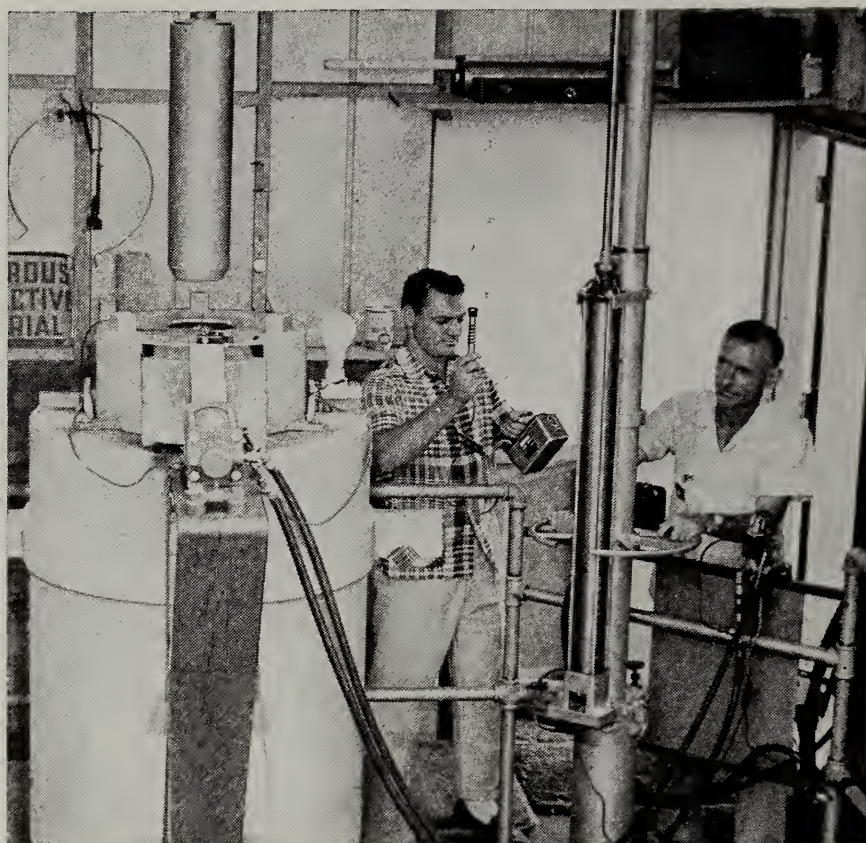


FIG. 27-4. Canister containing about 3 quarts of screw-worm pupae being lowered into Co^{60} source. About 6 minutes' exposure is required to give 7500 r in this source.

of Florida. The eradication of the species from the island by systematic weekly releases of males sexually sterilized by gamma rays [Baumhover et al. (30)] is one of the most interesting events in recent entomological history. This species was completely eliminated from the 170-square-mile island and has not been reintroduced up to the present time.

In the Curaçao test male screw-worm flies were sterilized by exposure to about 7500 r in a cobalt-60 unit (Fig. 27-4) and released at the rate of about 400 per square mile per week over a period of several months. The natural population of male flies was estimated to be about 100 to 200 per square mile at the start of the test. The sterile males caused a rapid decline of native flies, which meant that the ratio of sterile to fertile males increased rapidly with successive releases. The screw-worm population decreased greatly in about 3 months, and complete eradication was achieved in 5 months.

Research has indicated that the sterile-male technique is feasible for elimination of the screw-worm in Florida and the other Southeastern states. Eradicating the species in Florida, where it overwinters, and preventing its reintroduction from the other Southeastern states would free these states of the pest and result in much economic gain to the livestock industry.

In 1957 the State of Florida and the United States Department of Agriculture joined in a multimillion-dollar project aimed at elimination



FIG. 27-5. Oriental fruit flies laying eggs on papaya. (Shown life-size.)

of this pest. Construction has been started on a rearing facility for the production of approximately 50 million screw-worm flies per week. Half this number will be males. The flies will be made sterile by exposure to cobalt-60 and then released over all of peninsular Florida. The rates of release will range from about 100 to 800 males per square mile, depending on the natural population. Distribution will be made by airplanes flying at predetermined swath widths. The live flies will be stored in small containers which automatically open before reaching the ground after discharge from the airplane. Releases will be on a weekly basis, continuing for two years or until the pest is eliminated.

The research on the screw-worm that culminated in its successful elimination from Curaçao stimulated work on other insects. Steiner and Christenson (31) reported on irradiation studies of the oriental fruit fly, *Dacus dorsalis* (Hendel), (Fig. 27-5), the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), and the melon fly, *Dacus cucurbitae* (Coq.). These fruit flies are known to mate frequently. Preliminary tests indicated that sterile males were unable to reduce fertility in normal females originally mated with normal males. Approximately 10 days' confinement of sterile males with normal females was required to prevent subsequent fertilization by normal males. Doses of 8400 r or less permitted some recovery of fertility in males after 30-50 days. Loss of sterility

such as this could not be duplicated in the screw-worm fly, which lives about 3 weeks. The degree of sterility in fruit-fly males irradiated with cobalt-60 in the late pupal stage at doses ranging from 2500 to 10,000 r was proportional to the dose. Females lost their ability to produce eggs as the dose increased. Although 10,000 to 12,000 r were required to cause complete sterility and prevent egg laying, male effectiveness in inseminating females was in inverse ratio to the dose. The most practical range appeared to be 8000 to 10,000 r. Despite multiple mating and regaining of male fertility, tests of populations in laboratory cages indicated that fertility was inversely proportional to the ratio of irradiated to normal flies.

The Mediterranean fruit fly can be reared economically in pans containing a carrot medium (Fig. 27-6). Biological and sterility studies suggest that the species meets the requirements for control by release of sterile males over infested areas. The species has the advantage of being attracted to artificial lures, which will make field studies on population densities more exact (Fig. 27-7). Pilot tests in Hawaii and other islands in the western Pacific area are being considered by the Entomology Research Division.

Sexual sterilization of white-pine weevils, *Pissodes strobi* (Peck), by exposure to gamma rays has been studied by Jaynes and Godwin (32). They exposed the weevils to gamma radiations of 5000, 10,000, and 20,000 r. This is also a multiple-mating species. The life span was progressively shortened by increasing doses, averaging about 12 days when exposed to 20,000 r, as compared with 33 days for the untreated groups. Feeding activity of the irradiated weevils was reduced by approximately one-half. Nonirradiated females mated with males exposed to 5000 r produced eggs only 0.3 percent of which hatched, and exposure to 10,000-20,000 r prevented hatching. Previously mated nonirradiated females mated with males irradiated with 5000 and 20,000 r produced eggs, with hatches of 0.4 and 13.9 percent. The authors stated that these figures indicate that sterilized males are able to negate previous insemination by a normal male at the low exposure but not at the high exposure. Apparently the high dose materially damages the sperm, so that the older sperm from fertile males present in the female spermatheca can successfully compete.

In another experiment by Jaynes and Godwin, in which 20 normal females and 20 irradiated males were caged together for 12 days, 808 eggs were obtained, but none of them hatched. The irradiated males were then replaced with 20 normal males; in the following 12 days the females produced 938 eggs, 81 percent of which hatched. Therefore, normal males may nullify insemination by irradiated males. The authors conclude that the use of sterile males to control natural populations would



FIG. 27-6. Rearing Mediterranean fruit flies in a carrot medium for radiation studies. (Note mature larvae in lower collecting tray.)



FIG. 27-7. Developing attractants for use in sterile-male field tests of the oriental fruit fly.

not be feasible. However, with multiple-mating females an excess of sterile males by a high ratio might substantially reduce the biotic potential of the natural population.

IV. RADIOACTIVE INSECTICIDES

27-3 Insect physiology and toxicology. Laboratory studies have been conducted with labeled insecticides and repellents applied to insects, plants, and animals, especially since 1950. The earliest physiological research was done by Campbell and Lukens (33), who experimented with radioactive lead arsenate in silkworms to study the permeability of the gut wall. All these studies have yielded valuable information on the effectiveness and safety of insecticides. Dozens of materials have been labeled, including the chlorinated hydrocarbons, organophosphorus compounds, plant-derived and inorganic insecticides, and fumigants. Several of them have been labeled in various positions in the molecule and with different radioisotopes. Most of the radioactive insecticides have been studied on one or more species of insects, and some of them, especially DDT labeled with carbon-14, have received attention by various workers.

Radioactive insecticides have been employed for residue measurements and analyses. The importance of insecticide residues in plant and animal tissues has been apparent for many years, but the public is becoming increasingly aware of possible dangers in consuming contaminated foods. The labeling of insecticides, especially the organophosphorus materials, together with use of chromatographic techniques to identify and measure the parent compound as well as the metabolic breakdown products, has proved extremely valuable in such studies. For many insecticides chemical methods of analysis are unknown or inadequate. Frequently chemical methods lack the specificity and sensitivity necessary to measure residues and to detect the chemical changes of the compound in the living tissues.

Dahm (34) did excellent work in bringing together available research findings on the absorption, distribution, and metabolism of radioactive insecticides on insects. He presented tabular information regarding the position of the labeled atom in the molecule, source of radioactivity, and references to preparation of the various materials. This well-illustrated and documented paper is especially recommended for students, researchers in entomology, and all interested people in related scientific fields.

The use of radioactive compounds in insect metabolism has been discussed by Fay (35). In addition to insect studies, his paper gives the major results obtained in applying carbon-14-labeled DDT to labora-

tory animals. He points out advantages and limitations of tagged insecticides in metabolism studies. One of the advantages is that distribution of the materials and the effect of type and duration of exposure on absorption rates can be followed in the bodies of test animals with a high degree of accuracy. Chemical and other analytical procedures can also be checked for accuracy. The chief limitation is that supplemental chemical techniques are needed to identify the composition of breakdown products. Another disadvantage is that an overdose of radiation may also affect normal metabolism.

From studies on the mode of action and metabolic breakdown with carbon-14-labeled DDT on several strains of resistant house flies, Perry et al. (36) concluded that the small amounts of metabolites other than DDT produced after extended intervals do not appear vital to the fly's survival. Lindquist and Dahm (37), working with DDT-carbon-14 on the Madeira roach, *Leucophaea maderae* (F.), found DDT and three unidentified metabolites. They found no evidence of DDT metabolites in the fifth-instar European corn borer, *Pyrausta nubilalis* (Hbn.).

In studies on the absorption and excretion of radioactive piperonyl butoxide, a synergist for pyrethrum, on Madeira roaches, Schmidt and Dahm (38) found that about 88 percent was absorbed in 3 days. Paper chromatographic analyses of fecal extracts showed that less than 50 percent of the radioactivity was from piperonyl butoxide, the remainder from unidentified water-soluble metabolites. Acree et al. (39) prepared phosphorus-32-labeled Bayer L 13/59 (0,0-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) and studied its absorption and distribution in the American cockroach, *Periplaneta americana* (L.). For some time after topical applications the insecticide was found widely distributed, but after 20 hours most of the radioactivity was in the gut.

There have been many studies on the metabolism of radioactive organophosphorus insecticides in plants. Casida (40) has discussed their biochemical reactions. Other papers—by Casida et al. (41), Bowman and Casida (42), Arthur and Casida (43), and Metcalf et al. (44)—present basic information on plant metabolism of insecticides. Metcalf (45) discussed the use of radiotracers in studies of systemic insecticides in plants, reviewing methods of study, application of tracer techniques, and uses of radioactive insecticides in residue analysis, with special reference to systemic insecticides. No further reference will be made to systemic insecticides on plants, but in a later section the use of radiotracers and effectiveness of systemics in animals will be discussed.

Metabolism and residue studies have been made of labeled insecticides applied directly to livestock. Insecticides such as malathion are non-systemic and not to be confused with materials that are insecticidally active after being absorbed into animal tissues. March et al. (46) studied

the fate of malathion (S-[1,2-bis(ethoxycarbonyl ethyl] 0,0-dimethyl phosphorodithioate) on Jersey calves. This insecticide is effective against ticks, mites, and other livestock insects. They sprayed 1 pint of 0.5-percent phosphorus-32-labeled malathion on the calves twice, 2 weeks apart, and found that it was rapidly absorbed, metabolized, and eliminated in the urine. Their radiometric analyses 1 and 2 weeks after the last application showed that the residues in all the organ tissues were in the form of water-soluble metabolites and degradation products, with no unchanged malathion present. However, in the hide 2.7 percent (3 to 18 ppm) remained as unchanged malathion and chloroform-soluble metabolites 2 weeks after the second spray. Total residues in the meat cuts were low, 0.05 to 0.15 ppm.

Using the same labeled insecticide as a spray on laying hens, March et al. (47) found that the highest concentration of radioactive material was excreted in the droppings in the first 24 hours and that most of it was in the form of water-soluble metabolites and degradation products. Less than 12 percent of the applied dose was absorbed and eliminated in 32 days, an indication that malathion is poorly absorbed through the skin. Small amounts of radioactive material were found in the tissues, mostly in the form of chloroform-soluble metabolites. These writers also found that when malathion was incorporated in the feed, about 60 percent was eliminated in the droppings in 2 to 4 days. The residues in the tissues were about 10 times that in the sprayed hens.

27-4 Systemic insecticides in animals. Systemic insecticides, especially for the control of cattle grubs, have had an appeal to growers, meat packers, and the general public, because this new approach to insect control is more effective and less costly than conventional treatments. By systemic insecticides we mean those that enter the body tissues and destroy insects in or on the animal, such as cattle grubs, flies, mosquitoes, ticks, and lice. The most promising use of systemics at present is for control of cattle grubs, although research may disclose materials and methods that will provide effective and practical ways to destroy blood-sucking insects.

In the experimental work with systemics for control of cattle grubs, the main objective has been to find and explore the usefulness of compounds that will destroy young grubs before they are visible in the back, thus preventing flesh and hide damage and improving health. Young grubs hatch from eggs laid on the animal's hair and migrate into the body, where they remain for about 9 months before showing up in the back. A single effective treatment administered early in this period would make grub control easier and more economical than with rotenone sprays, which must be applied two or three times during the winter and spring.

Insecticides may act systemically when applied in different ways. They may be administered orally as a drench or bolus, injected subcutaneously or intramuscularly, sprayed on the skin, or incorporated with the feed. Some materials may kill insects when given via the stomach but not when applied externally, or vice versa. Others may be systemically active when applied in several ways. Thus far only one or two experimental materials have shown systemic activity when given intramuscularly.

One of the first organophosphorus compounds to show some systemic activity was Bayer L 13/59. This insecticide was effective in destroying grubs after they reached the back, but seemed to have little activity on those in the gullet and other tissues. Robbins et al. (48) reported on the oral administration of phosphorus-32-labeled L 13/59 in a lactating cow. A dose of 25 mg/kg of body weight was rapidly absorbed and metabolized by the cow and eliminated in the urine. Sixty-six percent of the dose was eliminated within 12 hours and only 0.26 percent of the radioactivity was unchanged L 13/59. The peak of radioactivity in the blood occurred between 1 and 3 hours, with a maximum of 15.1 μg -equivalents per milliliter. The exudate in the cattle grub cysts contained more radioactivity than the grubs themselves. The fact that the cattle grubs contained only the small amount of 1.03 μg -equivalents per gram is of interest. The maximum in the cyst exudates was 7.7 μg -equivalents per gram at 6 hours after treatment.

A major break-through in research with systemics for cattle grub control was made in 1954, when workers at the Corvallis, Oregon, and Kerrville, Texas, laboratories of the United States Department of Agriculture, found that Dow ET-57 [0,0-dimethyl 0-(2,4,5-trichlorophenyl) phosphorothioate] destroyed the young larvae within the host when given orally several weeks or months before they appeared in the back (49-51). This was the first insecticide ever found that would destroy young grubs before they made holes in the hide or appeared as lumps in the back.

The compound was labeled with phosphorus-32 by C. C. Roan and associates at Kansas State College and distributed for studies on the uptake of the insecticide by the grubs, residues in cattle tissue, and rates of absorption, degradation, and elimination by animal hosts. Robbins et al. (52) administered the radioactive material orally to cattle at the rate of 100 mg/kg. They found that 3 hours after treatment the material appeared in the blood at a rate of 1 μg -equivalent per milliliter, and that after 12 hours 22.8 μg -equivalent per milliliter was present. Paper chromatography of pooled Skelly B extracts of the blood demonstrated about 98 percent of the insecticide to be unchanged ET-57 and the remainder probably the oxygen analogue. During an 11-day period



FIG. 27-8. Making radioactive contamination measurements after a steer had been treated with P^{32} -labeled Dow ET-57.

about 86 percent of the administered dose was accounted for in the urine, the maximum amount between 6 and 30 hours. Chromatographic analysis showed only trace amounts of the parent compound, indicating rapid and extensive degradation. After 14 days the omental fat contained 6.9 ppm of unchanged ET-57, as determined by partition chromatography and bioassay.

Simultaneous work by Kaplanis and Hopkins (53) showed similar results, except that the omental fat samples were taken 3 days after treatment and they contained 50 ppm of unchanged ET-57 (Fig. 27-8). This and other work suggested that 60 days should elapse between treatment and slaughter of the animals. In September 1957 this insecticide was approved and recommended for use by the livestock grower on beef cattle only.

These authors also found that when screw-worms were allowed to feed on animals, the radioactivity in the larvae at the third hour amounted to 0.26 μg -equivalent per gram, but by the ninth hour it had increased to 5.21 μg -equivalent per gram. This is less than 0.1 μg -equivalent per larvae.

When stable flies were allowed to suck the blood of treated animals, 82 to 100 percent of them were killed during the first 36 hours and the mortality reached a peak at 8 hours. There was a close correlation between the fly mortality and the radioactivity of the Skelly B extractable material from the blood.

Preliminary work at the Kerrville, Texas, laboratory, in which animals were slaughtered at various times after treatment, showed some of the grubs encapsulated but dead in the gullet tissue. Much research will be necessary to determine how the insecticide or the metabolites destroy the grubs. It is possible that direct kill is not effected, but rather a disturbance to the insect's enzymes regulating migration or other activities. For example, the inhibition of the collagen-collagenase system, which Linert and Thorsell (54) found to play a part in the forward movement of grubs in cattle tissue, could well result from the use of systemic insecticides.

Another compound, Bayer 21/199 [0-(3-chloro-4-methylumbelliferone) 0,0-diethyl phosphorothioate], has the unique property of acting systemically against certain insects when applied as a spray [Brundrett et al. (55)]. When applied in this manner it is effective against cattle grubs several weeks or months before they encyst in the back, but has little action when given orally. It has some systemic action on bloodsucking stable flies and screw-worms but is a highly effective contact insecticide. In experimental work care must be taken to prevent contamination of the skin areas used for testing the bloodsucking insects in order to distinguish between systemic and contact effectiveness.

The use of phosphorus-32-labeled Bayer 21/199 was reported by Robins et al. (56). Two animals were sprayed in metabolism cages, one with 1 liter of 1.5 percent emulsion and the other with the same concentration in a wettable-powder suspension. Only low levels of radioactive compounds were found in the blood of either animal, the maximum occurring on the sixth day. The radioactivity in the emulsion-treated animal was about four times that in the one given the suspension. Only small amounts of radioactivity were detectable in the urine, with the peak amount appearing on the sixth day. Paper chromatographic analyses of samples taken on the fifth and sixth days showed only trace amounts to behave like unchanged Bayer 21/199. The compound appeared to be sparingly absorbed by the skin, with about 2.4 to 6.3 percent of the total applied dose being accounted for in the urine of the two animals after 2 weeks. At this time only very low levels of organo-soluble compounds which behaved like Bayer 21/199 were present in the tissues, but a large amount of unchanged material remained on the skin and hair at the end of 2 weeks.

In similar experiments with this compound, Kaplanis et al. (57) found low levels of radioactivity in the blood and urine and a large amount of unchanged insecticide on the hair as long as 6 weeks after treatment (Fig. 27-9). The maximum radioactivity of 0.21 μ g-equivalent per milliliter in the blood of one animal occurred on the third day. This work indicates that Bayer 21/199 is poorly absorbed and rather rapidly elimi-



FIG. 27-9. Applying P^{32} -labeled Bayer 21/199 as a spray through a special brush device.

nated. The small amount absorbed through the skin is apparently metabolized to a compound that is highly toxic to young cattle grubs in the tissues. Since the orally administered insecticide is apparently of a low order of effectiveness against grubs, it is concluded that the digestive tract either does not produce the toxic metabolite or destroys the action of the insecticide.

V. SUMMARY

Insects have always been of tremendous importance to man. They reduce crop yields, increase cost of production, and lower quality. They attack stored food, clothing, household furnishings, buildings, and livestock. They carry diseases of man and animals. New problems, such as resistance to certain insecticides, intensify the importance of further research on the control and biology of insects. The use of radioisotopes is having a stimulating and strengthening influence on many phases of entomological research. Biological studies with radioisotopes as tracers in dispersal and flight studies are helping us find the weak links at which to aim control measures. Tracers even may be used to evaluate effectiveness of control. They may be used in insect-physiology studies, in which a labeled chemical is traced during its absorption, metabolism, and excretion. A similar technique permits tracing the course of a systemic

insecticide in animal and plant tissues. Such a technique also aids in evaluating insecticide residue hazards.

Radiations have already been used to eradicate the screw-worm by means of the sterile-male technique. Subject to certain biological considerations, this may have possibilities in controlling other insect pests. Ionizing radiations can also be used to kill insects, but for this they may have limited application, since the lethal radiation dose for insects may be 100 times as much as that for man. However, this radiation has definite promise in treatment of bulk grain, and also in preventing the introduction of various insect pests inside fruit at quarantine inspection stations.

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Part 9

TRACER APPLICATIONS

- 28 Facets and Fashions in Physiological Tracer Experiments

by C. W. SHEPPARD

- 29 Intermediary Metabolism

by J. ASHMORE, M. KARNOVSKY, and
A. B. HASTINGS

- 30 Metabolism of the Major Mineral Elements of the Animal Body

by G. R. MENEELY, W. L. ALSOBROOK,
J. M. MERRILL, O. J. BALCHUM,
R. L. WEILLAND, and C. O. T. BALL

- 31 Photosynthesis

by M. CALVIN

